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From: Ford, Vanessa
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Please supply the following:

SO Poultry Science, (1997) Vol. 76, No. 5, pp. 677-682.

SO VETERINARY RECORD, (16 JAN 1993) Vol. 132, No. 3, pp. 56-59.

SO Journal of Parasitology, (1992) Vol. 78, No. 5, pp. 906-909.

SO AVIAN PATHOL, (1986) 15 (2), 271-278.

SO ACTA PARASITOL POL, (1976 (RECD 1977)) 24 (11-19), 103-117.

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Art Unit:1645

that nematode parasites have not been reported from *A. rufa*. The wet, relatively mild climatic conditions of the habitat, the fossorial habit and ancient lineage of *A. rufa*, and the direct life cycle of some parasitic nematodes would appear to make this host an ideal candidate for these parasites.

E. W. Baker and E. E. Wehr, USDA, and W. H. Lawrence, Weyerhaeuser Timber Company, assisted with identification of mites, cestode cysticerci, and fleas, respectively.

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Experimental Infections of *Eimeria alpacae* and *Eimeria punoensis* in Llamas (*Lama glama*)

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ABSTRACT: Four llamas (*Lama glama*) ranging in age from 1.5 yr to 7 yr each were inoculated orally with 10,000 (n = 2) or 50,000 (n = 2) sporulated oocysts of *Eimeria alpacae* (25%) and *Eimeria punoensis* (75%). The prepatent period for *E. alpacae* was 16–18 days, and it was 10 days for *E. punoensis*. Patent periods for *E. alpacae* and *E. punoensis* were approximately 9 days and 24 days, respectively. Although large numbers of oocysts were present in feces, no clinical sign of coccidiosis was observed. Based on this experiment, *E. alpacae* and *E. punoensis* at the numbers given are not likely pathogenic in healthy llamas older than 1 yr.

At least 4 species of *Eimeria* have been reported to infect llamas (*Lama glama*): *E. alpacae, E. lamae, E. macusaniensis,* and *E. punoensis* (Rickard and Bishop, 1988; Cheney and Allen, 1989). Reports of coccidial infections in llamas

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and Pathology, College of Veterinary

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are few, and specific biological information is incomplete. Previous reports have pertained principally to prevalence and sporulation times (Guerrero, Alva, Bazalar, and Tabacchi, 1970, Guerrero, Alva, Leguia, and Bazalar, 1970; Rickard and Bishop, 1988), with descriptions limited to recovery of *Eimeria* spp. oocysts from alpacas (*Lama pacos*) (Guerrero, 1967). Presently, no data exist regarding the prepatent period of *E. alpacae* and *E. punoensis* in llamas, and little is known concerning pathogenicity. The purpose of this study was to determine the prepatent and patent periods and evaluate the pathogenicity of *E. alpacae* and *E. punoensis* in 4 experimentally infected llamas.

Six llamas, 5 donated to Washington State University, Pullman, Washington, and 1 born at the University, were utilized in the study. The 5 donated llamas were from different farms in eastern Washington. Llama number 1 was a 1.5-yr-old gelding born at Washington State University; llama number 2 was a 7-yr-old gelding; llama number 3 was a 1.5-yr-old gelding; llama number 4 was a 5-yr-old female, the dam of llama no. 1; llama numbers 5 and 6 were 1-yr-old males. Llamas 1–4 were maintained on pasture and supplemented with alfalfa hay. Llamas 5 and 6 were housed indoors on a concrete floor with straw bedding and also fed alfalfa hay.

The initial coccidia inoculum was prepared from *Eimeria* spp. oocysts collected from the feces of a naturally infected llama submitted to the College of Veterinary Medicine at Washington State University, Pullman, Washington. Oocysts were concentrated by breaking the fecal pellets apart, mixing in a container of water, and filtering the mixture through 2 sieves with 500- and 250-μm openings. This sediment was mixed with 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and aerated in a 4-L flask at room temperature (21 C) for 16 days, at which time more than 80% of the oocysts had sporulated. The inoculum was washed by allowing the oocysts to settle for at least 1 hr in 800-ml glass beakers, decanting two-thirds of the supernatant portion, and adding more water. This procedure was repeated several times until the supernatant portion was clear. The inoculum contained approximately 75% *E. punoensis* and 25% *E. alpacae*.

On day 0, approximately 50,000 (llamas number 1, 2) or 10,000 (llamas number 3, 4) sporulated oocysts were administered orally with a dosing syringe. Llamas number 5 and 6 were uninoculated controls and did not receive oocysts. Llamas were observed for signs of disease daily.

TABLE I. Numbers of *Eimeria alpacae* and *Eimeria punoensis* oocysts per gram of feces recovered from 4 experimentally inoculated llamas.

Experimental day	50,000 oocysts		10,000 oocysts	
	Llama 1	Llama 2	Llama 3	Llama 4
0	0	0	0	0
7	0	0	NS*	NS
8	0	0	0	0
9	0	0	0	0
10	61†	1,086‡	1,880†	348†
11	260†	8,800‡	7,970†	6,945†
14	1,168†	10,305‡	3,096†	NS
16	5,175†	9,115‡	510†	2,300†
		1,965‡	40‡	660‡
18	470†	1,880‡	370†	540†
	720‡	9,720‡	250‡	3,110‡
22	115†	5,160†	810†	3,780†
	930‡	4,720‡	915‡	4,905‡
24	34†	15†	195†	2,230†
	14‡			1,670‡
28	105†	308†	144†	5,421†
31	10†	2,106†	0	315†
35	0	1,496†	0	0

* NS, no sample.
† *Eimeria punoensis*.
‡ *Eimeria alpacae*.

Fecal samples were collected from the rectum on experimental day 0, most days thereafter beginning on day 8 until the prepatent period was determined, and then approximately every 4 days until day 35 postinoculation (PI) (Table I). Microscopic examination of 1 g of feces from each llama for the presence of coccidial oocysts was conducted utilizing a standard sugar flotation technique (specific gravity = 1.27). Oocysts were viewed using a 40× objective and measured with an ocular micrometer.

Coccidial oocysts were not detected in the feces of any of the 6 llamas until day 10 PI. On day 10, *E. punoensis* oocysts were recovered from all 4 inoculated llamas (Table I). The mean size of unsporulated oocysts was 19.8 μm × 16.6 μm (n = 100). *Eimeria alpacae* oocysts were present in the feces of llamas number 2–4 on day 16 PI and llama 1 on day 18 PI (Table I). The mean size of unsporulated oocysts was 26.4 μm × 20.4 μm (n = 100).

Maximum numbers of *E. punoensis* oocysts per gram of feces occurred on day 11 PI for llamas number 3 and 4 (7,970 and 6,945, respectively), day 14 PI for llama number 2 (10,305), and day 16 PI for llama number 1 (5,175). Maximum numbers of oocysts of *E. alpacae* occurred

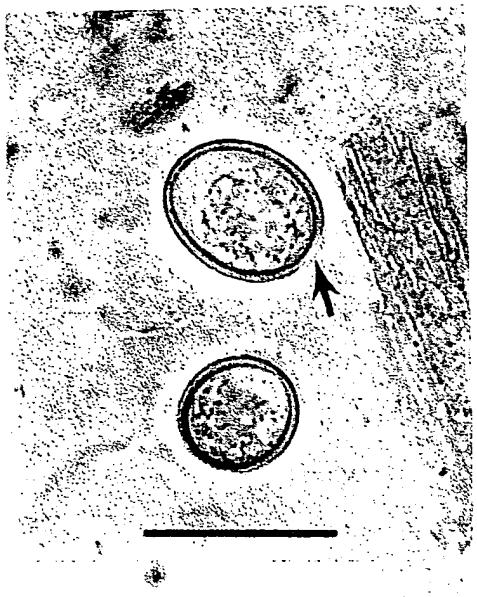

FIGURE 1. Unsporulated oocysts of *Eimeria punoensis* with distinct micropylar cap (arrow) and *Eimeria alpacae*. Scale bar, 30 μm.

on day 18 PI for llama number 2 (9,720) and day 22 PI for llamas number 1, 3, and 4 (930, 915, and 4,905, respectively). Oocysts of either species were not recovered from fecal examination of llama number 3 beyond day 28 PI, or from llamas number 1 and 4 on day 35 PI. *Eimeria punoensis* oocysts were recovered from feces of llama number 2 on day 35 PI, the last day fecal samples were collected. Oocysts of *E. alpacae* were last recovered from feces of the 4 inoculated llamas on day 24 PI. Oocysts were not detected in the feces of llamas number 5 and 6 (uninoculated controls) throughout this study.

The patent period of *E. alpacae* in this experiment was approximately 9 days, and for *E. punoensis* it was approximately 22–26 days (3 llamas). Llama number 2 continued to pass *E. punoensis* 26 days after oocysts first appeared; no further fecal sample was collected. A 10-day prepatent period for *E. punoensis* was determined, as fecal examinations of all 4 inoculated llamas were negative on days 8 and 9 PI and positive on day 10 PI. The prepatent period for *E. alpacae* was approximately 16–18 days, although samples were not collected on day 15.

Descriptions given by Guerrero (1967) of *E. alpacae* and *E. punoensis* in alpacas were in most respects similar to features observed in llamas in the present study (Fig. 1). He reported the mean size of sporulated oocysts of *E. alpacae* as 24.1 μm × 19.6 μm (n = 55). Our mean size of 100 unsporulated oocysts was slightly larger at 26.4 μm × 20.4 μm. The mean size of 58 *E. punoensis* sporulated oocysts reported by Guerrero (1967) was 19.9 μm × 16.4 μm, nearly identical to the 19.8 μm × 16.6 μm mean size of 100 unsporulated oocysts reported herein. It is known that the size of oocysts varies and is dependent upon the stage of patency, the number of oocysts present within the host, and the individual animal infected (Joyner and Long, 1974). In this experiment, unsporulated oocysts were measured from fecal samples of all 4 inoculated llamas from 1 to 3 days after the samples were collected, and the data were combined to arrive at the mean oocyst size reported.

Although micropylar caps are present on both species of coccidia, Guerrero (1967) stated that the micropylar cap of *E. punoensis* was indistinct and sometimes difficult to see, whereas *E. alpacae* has a distinct cap. We agree with this description of the micropylar caps, because the cap of *E. alpacae* usually was recognizable, and we experienced difficulty in observing the micropylar cap of *E. punoensis*.

No sign of disease was observed throughout the experiment in any of the inoculated llamas. Fecal samples remained firm and pelleted, with neither diarrhea nor blood detected. A lack of clinical signs of coccidiosis in this study agrees with previous observations of coccidia in llamas. Rickard and Bishop (1988) reported a high prevalence of *E. lamae* among llama crias with no apparent clinical disease, and they stated that coccidia may not be as pathogenic for llamas as they are for alpacas. *Eimeria lamae* is considered to be pathogenic for alpaca crias (Guerrero, Alva, Bazalar, and Tabacchi, 1970), and *E. macusaniensis* has been associated with enteritis in a llama (Schrey et al., 1991) and is reported to be pathogenic in alpacas (Guerrero, Alva, Leguia, and Bazalar, 1970). Cheney and Allen (1989) reported that young llamas may show signs of clinical coccidiosis, primarily diarrhea, but that most coccidia infections in llamas are asymptomatic.

We report for the first time the prepatent periods for *E. alpacae* and *E. punoensis* in llamas. In this experiment we inoculated 4 llamas ranging in age from 1.5 yr to 7 yr with 2 concentration

levels of oocysts of *E.* Signs of disease were n llamas, and no differen status or fecal consist ceiving the different co Our data indicate that *sis* at the numbers giv healthy llamas greater

We thank Kriss Hoff and Kirk Johnston for experiment.

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Equine Protozoa *Sarcocystis neu*

D. E. Granstrom, O. Alva Center, Department of Vet Desarrollo Agropecuario, Pa Sciences Institute, Agricult

ABSTRACT: Schizonts of identified microscopically i spinal cord sections from that exhibited clinical sign tis (EPM). Spinal cord ho amanian horse with EPM layers of cultured bo Intracytoplasmic schizont ranged in rosette forms su body first were observed asites divided by endopol Schizonts from each horse *zi* antiserum in an immun

Equine protozoal my an often debilitating (CNS) disease of the ho in horses native to N (Barros et al., 1986; Fa *cystis neurona*, the eti cently was cultured fr horses from New Yor Daft, and Dubey, 199

levels of oocysts of *E. alpacae* and *E. punoensis*. Signs of disease were not observed in any of the llamas, and no difference was observed in health status or fecal consistency between llamas receiving the different concentrations of inoculum. Our data indicate that *E. alpacae* and *E. punoensis* at the numbers given are not pathogenic in healthy llamas greater than 1 yr old.

We thank Kriss Hoffman, Brooke Cummings, and Kirk Johnston for their assistance with the experiment.

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Equine Protozoal Myelitis in Panamanian Horses and Isolation of *Sarcocystis neurona*

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ABSTRACT: Schizonts of *Sarcocystis neurona* were identified microscopically in hematoxylin–eosin-stained spinal cord sections from 2 native Panamanian horses that exhibited clinical signs of equine protozoal myelitis (EPM). Spinal cord homogenate from a third Panamanian horse with EPM was inoculated onto monolayers of cultured bovine monocytes (M617). Intracytoplasmic schizonts containing merozoites arranged in rosette forms surrounding a central residual body first were observed 13 wk postinoculation. Parasites divided by endopolygeny and lacked rhoptries. Schizonts from each horse reacted with *Sarcocystis cruzi* antiserum in an immunohistochemical test.

Equine protozoal myeloencephalitis (EPM) is an often debilitating central nervous system (CNS) disease of the horse. It has been reported in horses native to North America and Brazil (Barros et al., 1986; Fayer et al., 1990). *Sarcocystis neurona*, the etiologic agent of EPM recently was cultured from 2 naturally infected horses from New York and California (Davis, Dafi, and Dubey, 1991; Davis, Speer, and Dubey, 1991; Dubey et al., 1991). In the present paper we describe EPM in 3 horses born and raised in Panama and report in vitro cultivation of *S. neurona* from 1 of them.

The affected horses were from adjoining farms located approximately 1.8 km above sea level on the Pacific side of the continental divide in northwestern Panama. Posterior ataxia had been observed in 7.5% (22/292) of the yearlings on 1 farm from 1985 to 1991. A 17-mo-old thoroughbred colt (horse 1), a 15-mo-old thoroughbred filly (horse 2), and a 2-yr-old thoroughbred filly (horse 3) developed posterior ataxia at different times. Each became progressively uncoordinated and was killed. The brain and spinal cord were removed from each and processed for histological examination. Hemorrhages were visible grossly on cut sections of spinal cord from each horse. Portions of several visible lesions in the gray and white matter from horse 3 were processed for tissue culture as described (Davis, Speer, and Dubey, 1991).